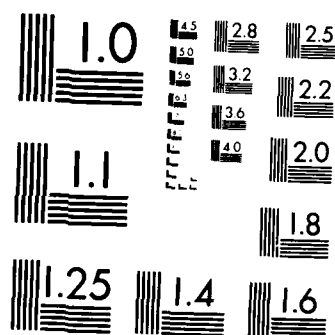


AD-A170 737 RAPID DIAGNOSIS OF ARBOVIRUS AND ARENAVIRUS INFECTIONS 1/1
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SCHOOL OF MEDICINE G H TIGNOR 31 JAN 83
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A serologic survey of equatorial Africa for antibodies in man to Crimean-hemorrhagic fever-Cong, Rift Valley fever, Ebola, Lassa fever, and Marburg viruses was continued. In Sudan, prevalence rates were Marburg 0.2%, CCHF 0.7%, Lassa 3.9%, RVF 4.6%, EBO-Zaire 8.2%, and EBO-Sudan 11.2%. Significant geographic clustering of antibody positive sera occurred for Lassa, EBO-Z and EBO-S viruses. Over 1000 sera from the Benue river basin in Nigeria which were collected by WHO in 1964-1965 have been tested. The prevalence rates are Congo 27%, RVF 17%,

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Continued 20.ABSTRACT

EBO-Z 6%, EBO-S 39% with 27% positive to Congo, and 29% positive to Lassa. An ELISA for CCHF virus has been developed. Human sera from Ethiopia and Senegal are still under study. From preliminary results, it appears that the rate of positive reactions varies with geographic region within each country.

RAPID DIAGNOSIS OF ARBOVIRUS AND ARENAVIRUS INFECTIONS BY IMMUNOFLUORESCENCE
ANNUAL REPORT

Gregory H. Tignor, Sc.D.

31 January 1983

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TABLE OF CONTENTS

| | |
|--|----|
| Title Page | 1 |
| Table of Contents | 2 |
| Summary | 3 |
| Foreward | 4 |
| Results | 5 |
| Serological survey in the Sudan | 5 |
| Table 1 | 6 |
| Table 2 | 7 |
| Figure 1 - Study Region in the Sudan | 8 |
| Figure 2 - Economic Activity in the Sudan Study Area | 9 |
| Figure 3 - Vegetation in the Sudan Study Region | 10 |
| Figure 4 - Sudan Study Area | 11 |
| Figure 5 - Antibody Positives in Nigeria | 12 |
| Senegal human serosurvey | 13 |
| Serosurvey in Ghana | 13 |
| Serosurvey in Ethiopia | 14 |
| Crimean-Congo hemorrhagic fever virus ELISA | 15 |
| Distribution List | 16 |



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SUMMARY

A serologic survey of equatorial Africa for antibodies in man to Crimean-hemorrhagic fever-Congo, Rift Valley fever, Ebola, Lassa fever, and Marburg viruses was continued. 587 sera from Sudan have been tested. The prevalence rates were Marburg 0.2%, CCHF 0.7%, Lassa 3.9%, RVF 4.6%, EBO-Zaire 8.2%, and EBO-Sudan 11.2%. Significant geographic clustering of antibody positive sera occurred for Lassa, EBO-Z and EBO-S viruses. For each virus, the northern provinces had little or no evidence of antibody, but the southern and southwestern provinces (bordering Central African Republic, Zaire, Chad and Uganda) had significantly higher rates (as high as 34% for EBO-S). The provinces located in central Sudan had varied prevalence rates. We have located a village in one of these central provinces with a 26% rate for EBO (Z and S). However, villages within 100 miles have rates less than 0%. Ebola positive reactions have also been found in sera from life-long residents of Senegal's lower Fleuve region in the Senegal River Valley. Antibody to Rift Valley Fever and Lassa viruses was also found in Senegal. Human sera from Ethiopia and Senegal have been screened on polyvalent spot-slides. Human sera from Ethiopia and Senegal have been screened on polyvalent spot-slides. The rate of positive reaction varies with geographic region. Over 1000 sera from the Benue river basin in Nigeria which were collected by WHO in 1964-1965 have been tested. The prevalence rates are Congo 27%, RVF 17%, EBO-Z 24%, EBO-S 50%, Lassa 29%, and Marburg 3%. Of the EBO positives, 6% react only with the Zaire strain, 39% react only with the Sudan strain, and 13% react with both.

Over 1000 sera from the Benue River basin have been screened on polyvalent slides. 985 have been tested on monovalent slides. The greatest proportion of positives are to Ebola virus strains Zaire (6%) and Sudan (39%) with 27% positive to Congo, and 29% positive to Lassa.

FOREWARD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Results during the fifth period of the contract (1 February 1982 to 31 January 1983). The present principal investigator has served since August, 1981.

Serological survey in the Sudan. (in collaboration with J. Meegan and T. Bucci, NAMRU-3). It is likely that many viruses which circulate in northern and southern Africa are disease problems in Sudan. Few studies have been undertaken in this geopolitically important country. During 1979, 1980, and 1981, over 3400 sera were collected from military recruits in Sudan. These represent collections from all areas of Sudan (age, birthplace, and district of residence are available for all). This survey is one phase of a long-term study to determine the impact of a number of virus infections on humans and animals in Sudan. In addition, since Sudan may act as a tunnel for the movement of viral disease from sub-Saharan Africa to Egypt and beyond, survey for other viruses may give clues as to what diseases to be alert for.

Twenty to forty sera from soldiers native to villages near each base where sera were collected were tested on polyvalent CRE2LM slides. Since these soldiers grew up and were stationed in the same area, we used them as an indication of which areas in Sudan were endemic for hemorrhagic fever viruses. Interestingly, only one sera of 55 collected in area near Khartoum was positive (for RVF virus) while rates of greater than 25% were seen in southern provinces. Since soldiers stationed in and around Khartoum had little chance of contracting the disease during military training, we considered sera from these soldiers are representative of the antibody prevalence rates in their native governorates, and included them in our studies of the distribution of these viruses in Sudan. Over 580 sera have been screened on polyvalent CRE2LM slides and most retested on monovalent slides. Table 1 gives the prevalence data for antibodies to each virus (refer to Figure 1). Significant geographic clustering of antibody positive sera occurred for Lassa, EBO-Z and EBO-S viruses. For these viruses, the northern provinces had little or no evidence of antibody, but the southern and southwestern provinces (bordering Central African Republic, Zaire, Chad and Uganda) has significantly higher rates. The provinces located in central Sudan had varied prevalence rates. The majority of endemic areas have a savanna type of vegetation; the major economic activity is grazing (Figures 2 and 3).

In the central province of Southern Kordofan, we have located one village (Muglad) with a high antibody prevalence rate for Ebola virus (Table 2). But villages in the same ecological zone within 100 miles (Figure 4) of Muglad show low antibody prevalence rates. If the prevalence rates do not change as we test additional sera from the control villages, this might be an excellent future study site for Ebola virus.

We studied the EBO antibody prevalence rates for troops native to a non-endemic area (El Obeid and Kadugli) (Figure 4 and Table 2) but stationed in either endemic or non-endemic areas. It appears that there are significantly higher antibody prevalence rates in the group of soldiers who trained in the endemic region. We are now testing more sera, and awaiting additional sera from the collection stored at NAMRU-3.

Table 1

| | | <u>Percent Positive</u> | | | | | |
|--------------------|-------------------------------|-------------------------|-------------|--------------|--------------|--------------|------------|
| <u>Province</u> | <u>Number sera tested</u> | <u>RVF</u> | <u>CCHF</u> | <u>EBO-Z</u> | <u>EBO-S</u> | <u>Lassa</u> | <u>MAR</u> |
| 1. E. Equatoria | 35 | 5.7 | 2.9 | 17.1 | 20.0 | 8.6 | 0.0 |
| 2. W. Equatoria | 51 | 5.9 | 0.0 | 25.5 | 21.6 | 2.0 | 2.0 |
| 3. El Buheyra | (8) | (12.5) | (0.0) | (0.0) | (12.5) | (12.5) | (0.0) |
| 4. Jungles | 30 | 13.3 | 0.0 | 0.0 | 10.0 | 0.0 | 0.0 |
| 5. Bahur El Ghazal | 62 | 8.1 | 1.6 | 16.1 | 27.4 | 16.1 | 0.0 |
| 6. S. Darfur | 33 | 3.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7. S. Kordofan | | | | | | | |
| Muglad area | 34 | 8.8 | 0.0 | 7.9 | 26.5 | 0.0 | 0.0 |
| non-Muglad area | 38 | 2.6 | 0.0 | 0.0 | 0.0 | 2.9 | 0.0 |
| 8. Upper Nile | 10 | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 9. N. Darfur | 33 | 0.0 | 0.0 | 0.0 | 3.0 | 0.0 | 0.0 |
| 10. N. Kordofan | 31 | 0.0 | 0.0 | 3.2 | 0.0 | 3.2 | 0.0 |
| 11. White Nile | 1 | - | - | - | - | - | - |
| 12. Blue Nile | 6 | - | - | - | - | - | - |
| 13. El Gezira | 15 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 14. Kassala | 31 | 3.2 | 0.0 | 3.2 | 0.0 | 0.0 | 0.0 |
| 15. Khartoum | 25 | 4.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 16. Nile | 16 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 17. Red Sea | 19 | 0.0 | 0.0 | 0.0 | 15.8 | 0.0 | 0.0 |
| 18. Northern | 20 | 5.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Table 2

| <u>Village</u> | <u>Number sera tested</u> | <u>Number positive (EB0-Z and EB0-S)</u> |
|----------------|---------------------------|--|
| Muglad | 34 | 10 |
| Dilling | 12 | 0 |
| Kadugli | 7 | 0 |
| El Fula | 8 | 0 |
| El Obeid | 18 | 1 |
| El Fasher | 30 | 1 |
| Nyala | 29 | 0 |
| Wau | 50 | 16 |

Figure 1
Study Region in the Sudan

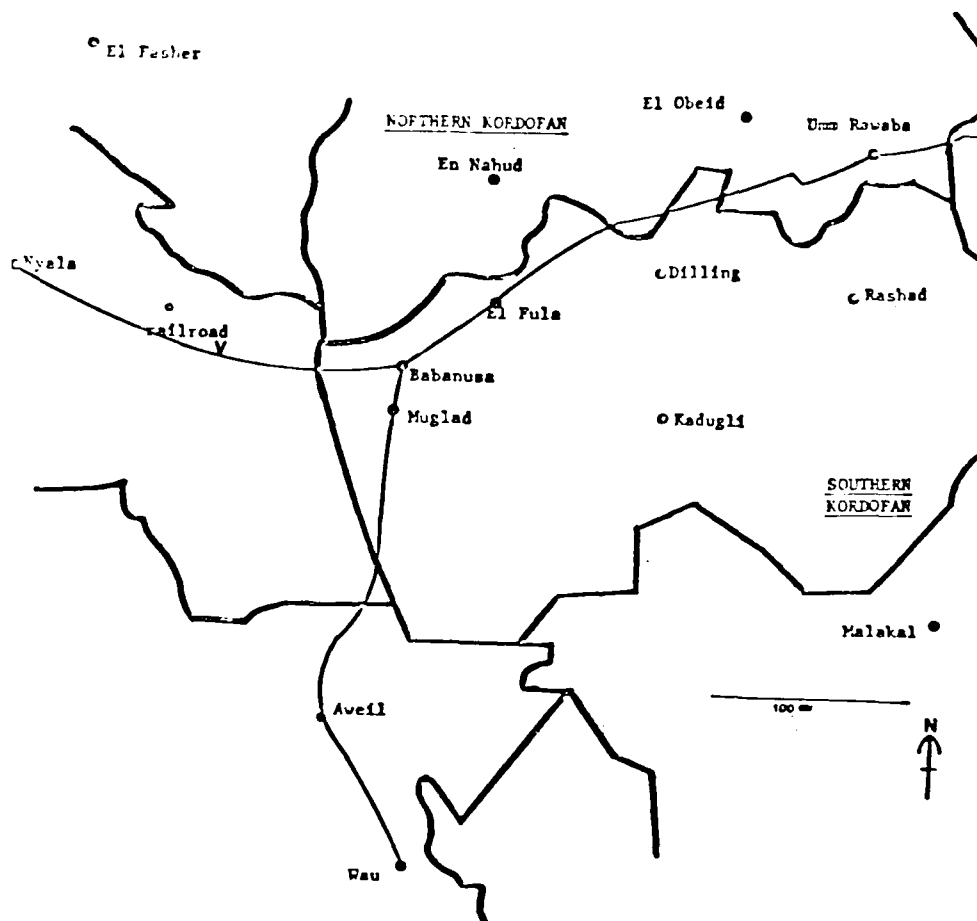


Figure 2

Economic Activity in the Sudan Study Area

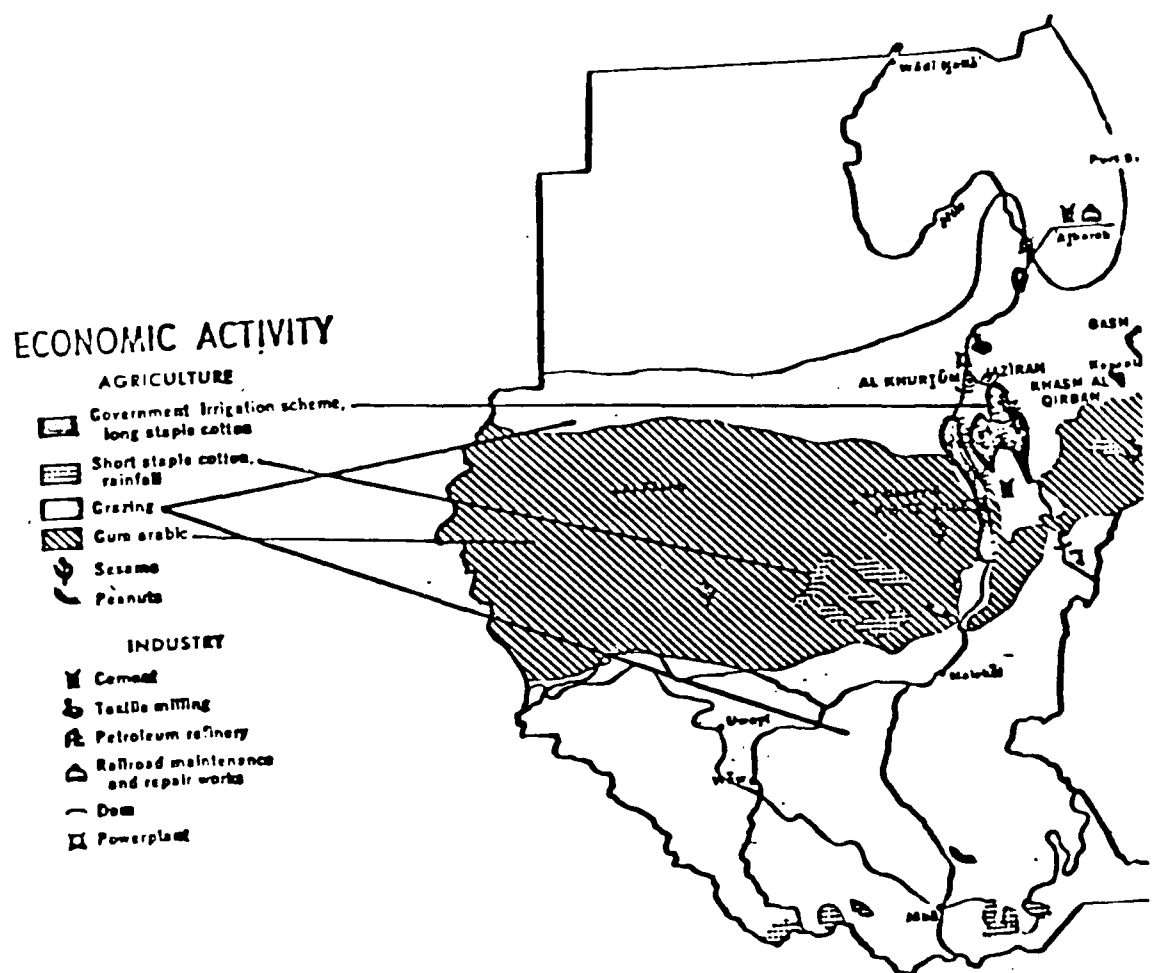


Figure 3

Vegetation in the Sudan Study Region

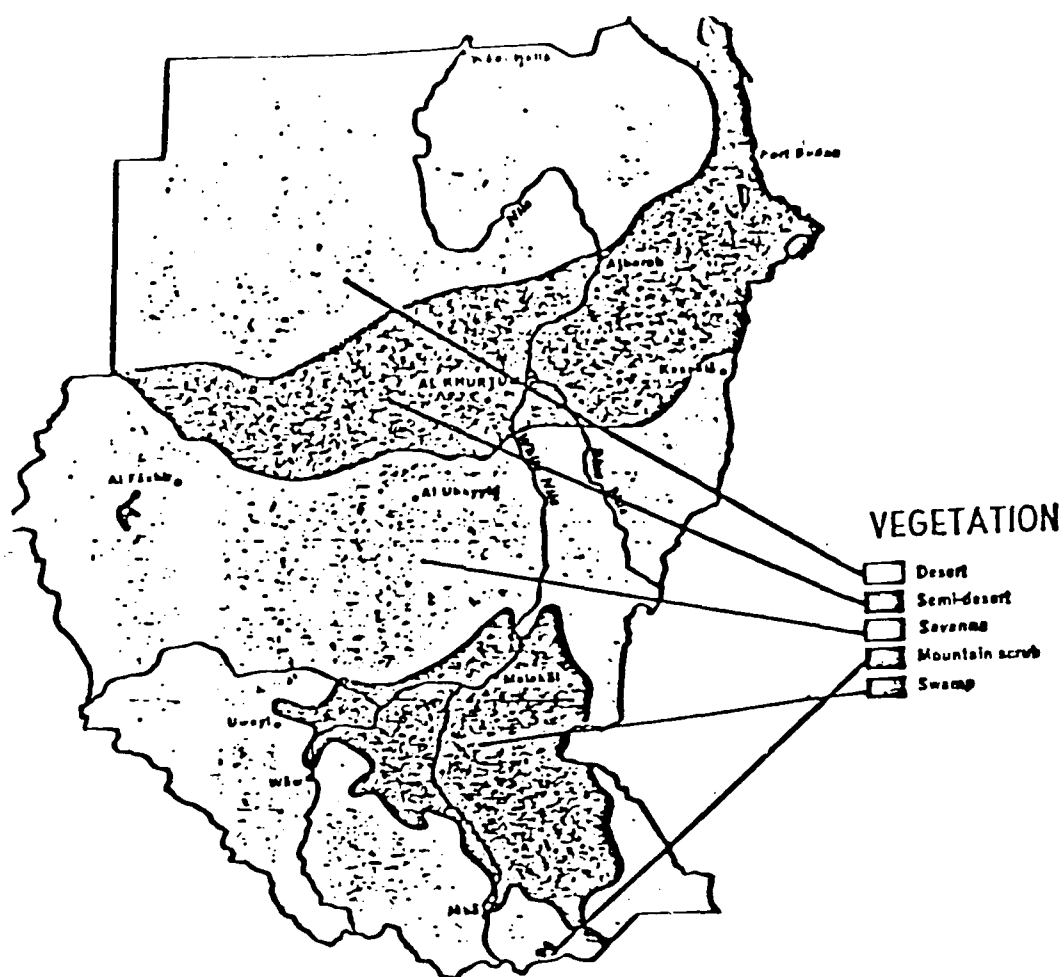
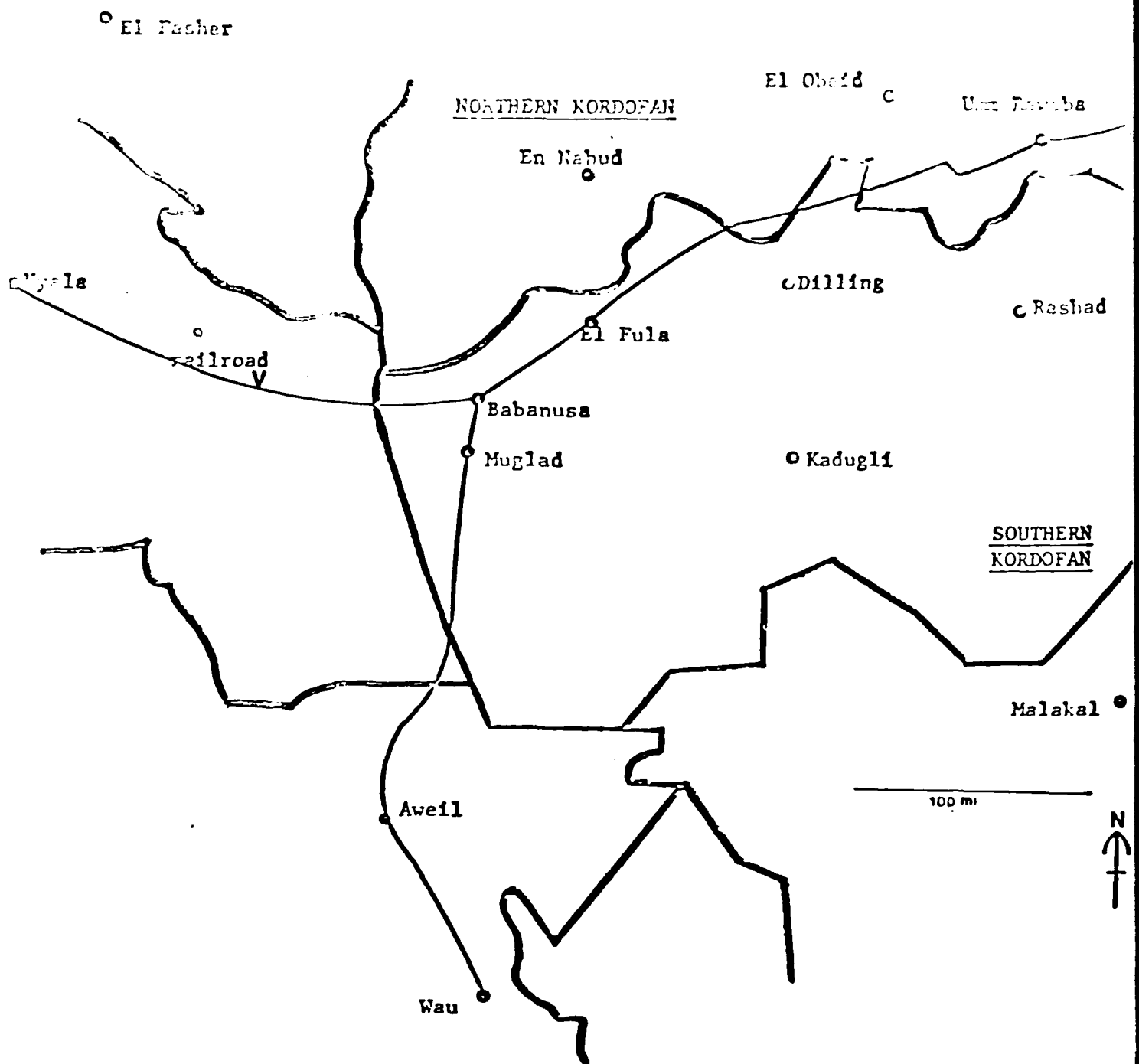
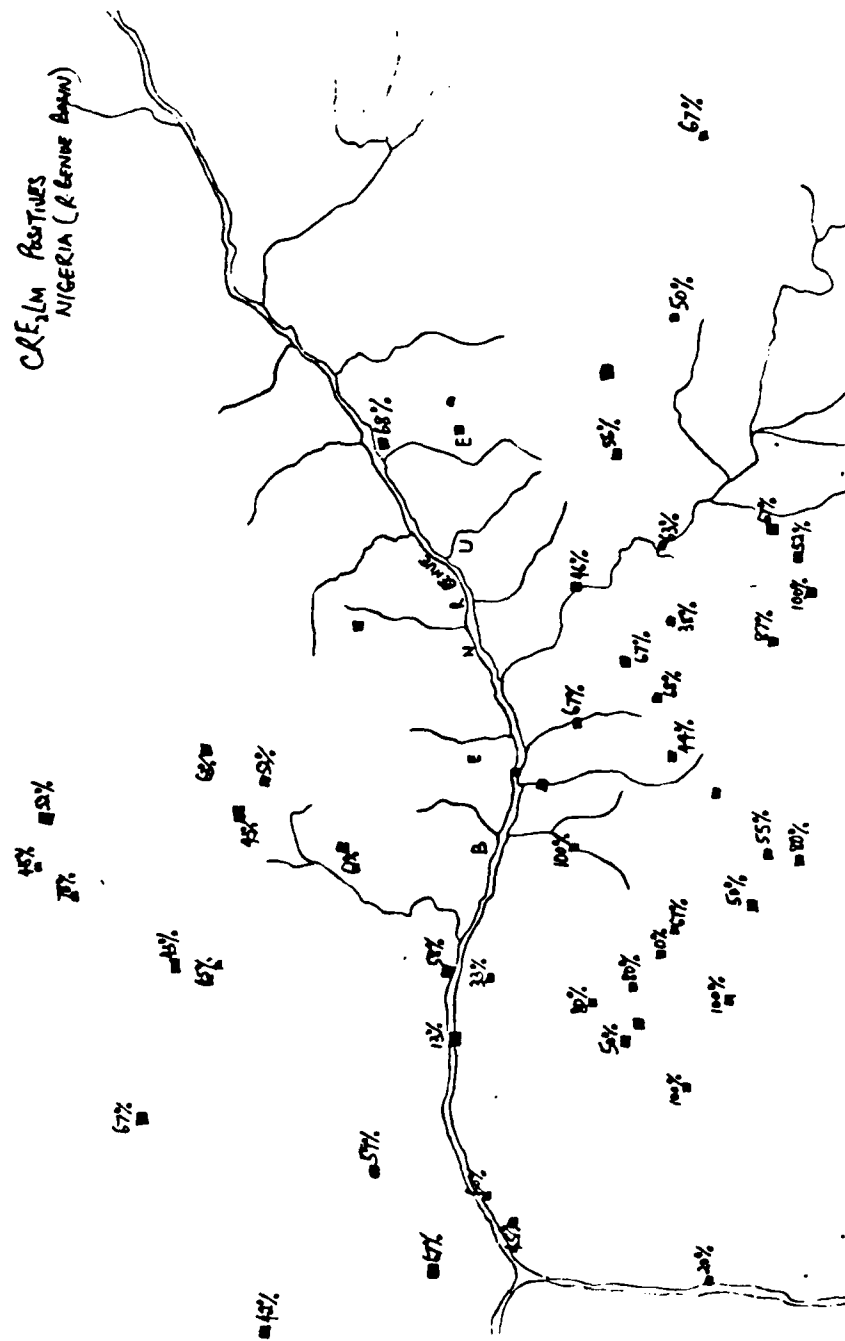


FIGURE 4 - Sudan Study Area



Antibody Positives* in Nigeria
a) Benue River Basin



*CRE₂LM Positives are
CCHE, RVF, EBOLA, LASSA, MARBURG

Senegal human serosurvey for Rift Valley fever, Ebola, Marburg, Lassa, and Crimean-Congo hemorrhagic fever

A serological survey of human sera collected in Senegal by a YARU team headed by W.G. Downs in 1977 was done using indirect immunofluorescence and spot-slides. The slides were provided by Dr. K.M. Johnson, CDC. Polyvalent slides were used to screen 283 sera for RVF, Ebola, Marburg, Lassa, and CCHF. Not all sera were tested for all viruses. Of 283 sera tested, 37 were positive to one or more antigens.

Six sera were positive with spot-slides containing all 5 viruses; 4 out of 5 of those tested for RVF were mono-specific for RVF. Only 4 of 31 positive to the polyvalent Ebola-Marburg-Lassa spot-slides were tested further.

The cumulative positive results obtained by J. Casals and G. Tignor so far indicated Ebola antibody in 6 persons (2 were children aged 5 and 8 years), Lassa antibody in one person, and RVF antibody in 4 persons. All of these persons claimed to be life-long residents of Senegal's lower Fleuve region in the Senegal River valley. This is the site where multi-national construction teams are about to start building a series of dams.

In view of the surprising finding of RVF antibody and the known cross-reaction by IFA with other phlebotomus fever group viruses, tests were done to determine the specificity of the reaction with 2 of the sera. The results indicated full specificity for RVF. In addition, PRNT performed by Dr. C.J. Peters, USAMRIID, confirmed the RVF positive reactions.

To date, we have not completed additional testing on this collection.

Serosurvey in Ghana

Two hundred forty-seven human sera collected in Ghana in 1975 were screened on ELM slides during the past year. We have found many positives with sera from individuals in the Eastern (42%) and Western (34%) regions and fewer positives from the Ashanti (18%) region. We have not yet screened sera from the Brong-Ahafo or the Northern regions.

Ghana positives on ELM slides by geographic region

| Region | Results | | |
|-------------|------------|--------|---------|
| | Positive | Tested | Percent |
| Eastern | 35 | 83 | 42 |
| Western | 35 | 102 | 34 |
| Brong-Ahafo | Not tested | | |
| Ashanti | 11 | 62 | 18 |
| Northern | Not tested | | |

Serosurvey in Ethiopia

We have screened 201 sera from the Ethiopian collection and all of these sera were tested on ELM slides.

Ethiopian survey results by region on ELM slides

| Region | Positive | Negative | Per Cent |
|---------------|----------|----------|----------|
| Assab | 3 | 17 | 15 |
| Blue Nile | 26 | 44 | 37 |
| Sidamo-Borena | 10 | 50 | 17 |
| Ogaden | 0 | 4 | 0 |
| L'Aquache | 14 | 33 | 30 |

The breakdown of positives by region shows more positives in the Blue Nile and Valle deL'Aquache regions than in the Assab, Sidamo-Borena regions. (Only 4 sera have been tested from Ogaden). However, within the Blue Nile region, 8 of 26 total positives (31%) come from two villages where each individual tested was positive. There were 25 villages sampled in the region. Similarly, in the Valle deL'Aquache, 8 of 14 total positives (57%) came from 2 villages. There were 12 villages sampled in this region. It appears from these preliminary data that there is a marked localization of virus activity within these broad geographic regions. Again, in the Sidamo-Borena region, 8 of 10 positives came from 3 villages of 25 sampled.

We have not finished testing this collection of sera.

We have screened 1008 sera from Nigeria on polyvalent slides; most of these (985) have been tested on monovalent slides. The results are given in the tabular material below.

| | CRE2LM | Congo | RVF | EBO-Z | EBO-S | Lassa | Marburg |
|-----------|--------|-------|-----|-------|-------|-------|---------|
| Positive | 602/ | 32 | 4 | 56 | 72 | 68 | 7 |
| Tested | 1008 | 120 | 23 | 237 | 149 | 254 | 202 |
| %Positive | 60 | 27 | 17 | 24 | 50 | 29 | 3 |

Monotypic EBOLA positives

| EBO-ZAIRE | EBO-SUDAN | BOTH |
|-----------|-----------|--------|
| 8/144 | 56/144 | 19/144 |
| 6% | 39% | 13% |

A map of the Benue river basis showing the distribution of CRE2LM positive is given in Figure 5. When testing is completed, the data will be analyzed further.

Crimean-Congo hemorrhagic fever virus ELISA

We have prepared antigen for the enzyme-linked immunosorbent assay using inactivated virus derived from potassium tartrate-glycerol gradients. The CCHF ELISA reliably detected antibodies in hyperimmunized mice, and when tested in preliminary studies of sera from residents of Greece, it correlated well with the HI and IF tests.

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